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Omega-3 fatty acids (fish-oil) and depression-related cognition in healthy volunteers

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Abstract

Omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation may be beneficial in the treatment of several psychiatric disorders, including depression. A small number of studies have suggested that there may also be cognitive and mood effects in healthy samples. The purpose of the present study was to investigate the effects of n-3 PUFA on depression-relevant cognitive functioning in healthy individuals. Fifty-four healthy university students were randomized to receive either n-3 PUFA supplements or placebo for 4 weeks in a double-blind design. The test battery included measures of cognitive reactivity, attention, response inhibition, facial emotion recognition, memory and risky decision-making. Results showed few effects of n-3 PUFAs on cognition and mood states. The n-3 PUFA group made fewer risk-averse decisions than the placebo

group. This difference appeared only in non-normative trials of the decision-making test, and was not accompanied by increased impulsiveness. N-3 PUFAs improved scores on the control/perfectionism scale of the cognitive reactivity measure. No effects were found on the other cognitive tasks and no consistent effects on mood were observed. The present findings indicate that n-3 PUFA supplementation may have a selective effect on risky decision making in healthy volunteers, which is unrelated to impulsiveness.

Key words

cognition; decision-making; depression; mood; omega-3 fatty acids

Introduction

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are long-chain polyunsaturated fatty acids found mainly in fish oil and include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Beneficial effects of n-3 FAs have been reported for a range of psychiatric disorders, including mood disorders, schizophrenia and dementia (Freeman, 2000; Young and Conquer, 2005). N-3 PUFAs may affect neuronal functioning through several mechanisms (see Parker, *et al.*, 2006), including decrease of the production of inflammatory eicosanoids from arachidonic acid; inhibition of the release of proinflammatory cytokines and increase of brain-derived neurotrophic factor. Furthermore, DHA has a role in membrane integrity and fluidity, affecting neurotransmission (Yehuda, *et al.*, 1999). Animal studies have shown that n-3 PUFAs deficiencies may affect

dopaminergic and serotonergic neurotransmission (De la Presa and Innis, 1999; Chalon, 2006). A reduction of serotonin release was found in the hippocampus of rats chronically deficient in n-3 PUFAs (Kodas, *et al.*, 2004). In humans, DHA correlated with the concentration of serotonin metabolites in cerebrospinal fluid (Hibbeln, *et al.*, 1998).

Supporting evidence for the effects of n-3 PUFAs has been reported for conditions characterized by impulsivity (reviewed by Hallahan and Garland, 2004). Two recent meta-analyses have evaluated the effects of n-3 PUFAs on depressed mood. Lin and Su (2007) concluded that there is a moderate effect of n-3 PUFA supplementation on depressed mood in patients with mood disorders. There was some evidence for a dose–response relationship, but publication bias was also considered likely. Appleton, *et al.* (2007) evaluated randomized controlled trials in various populations, including not only psychiatric

populations but also patients with angina and healthy samples. They concluded that there is little evidence that n-3 PUFAs improve mood. Although these authors also found a medium effect size in trials involving patients with mood disorders, they argue that results are hard to interpret because of the heterogeneity of interventions, measures and populations, and because of probable publication bias. In almost all trials, patients received n-3 PUFAs in addition to antidepressant medication.

Investigation of healthy samples avoids possible confounders such as varying remission status and adjunctive treatment. Although subjective improvement of mood is not to be expected from short-term treatment with n-3 PUFAs in healthy individuals, cognitive functions may be affected. Research on the effects of anti-depressants has shown that cognitive effects may occur independently from symptom changes and that these effects may appear in healthy individuals even after one dosage. For instance, a single dose of citalopram improved accuracy and speed of recognition of facial expressions of fear without affecting mood (Harmer, *et al.*, 2003; Browning, *et al.*, 2007). These findings suggest that cognitive tests may be more sensitive to the early effects of antidepressants than subjective mood states. The effects were also selective as they were only evident in tasks involving emotional information processing (review in Merens, *et al.*, 2007).

Research on the effects of n-3 PUFAs supplements in healthy subjects has been quite limited. Hamazaki, *et al.* (1996) found that 1.5–1.8 g DHA/day for 3 months prevented a rise of aggression during mental stress periods in healthy students. Fontani, *et al.* (2005) investigated the effects of 5-week supplementation of n-3 PUFAs (1.6 g EPA, 0.8 g DHA) versus placebo. The n-3 PUFA group had improved self-reported anger, anxiety, fatigue, depression, confusion and vigour scores and faster reaction times on a go/no-go task and a sustained-attention task. EEG recordings showed greater amplitudes of the negative wave preceding and of the positive peak following the stimuli (CNV and P3) during the go/no-go task. All participants in this trial were regular exercisers (>4 h/week), which reduces generalization. More importantly, the reported effects appear rather small and inadequately analysed. Paired *t*-tests showed significant changes in the n-3 PUFA group and not in the placebo group; however, analysis of variance tests were not conducted and the data for the placebo group were not reported. Hence, we cannot be sure that the improvements in the n-3 PUFA group were significantly larger than changes in the placebo group.

The purpose of the present study was to examine the effects of n-3 PUFAs on depression-related cognition in healthy volunteers. On the contrary, with Fontani, *et al.* (2005), participants were not selected on the basis of physical fitness and tests were chosen that are relevant to depression. The test battery measured attention to emotional stimuli, response inhibition, facial expression recognition and risky decision-making. The effects on mood and neutral information processing were also examined, allowing us to compare our findings with Fontani, *et al.* (2005); however, the primary outcome measure was emotional information processing. We selected a number of

cognitive tests, which in previous studies have been shown sensitive to neurotransmitter manipulations in healthy samples (Merens, *et al.*, 2007) or to depression vulnerability (Austin, *et al.*, 2001). For example, subjects' processing of reward and punishment cues when making risky choices are partly dissociable following manipulations of serotonin (Rogers, *et al.*, 2003), noradrenaline (Rogers, *et al.*, 2004), dopamine (Scarna, *et al.*, 2005) and cannabinoid systems (Rogers, *et al.*, 2007).

Materials and methods

Participants

Eligible participants were healthy individuals with a body mass index (BMI) between 18 and 27, who maintained a regular diet containing fish not more than once a week. Exclusion criteria were a current episode of major depression (current = month before the study), current or previous psychosis, current substance abuse or previous substance dependence, any illness requiring medication, smoking or current use of soft drugs, hard drug use (lifetime) and more than three alcoholic consumptions/day.

Dietary supplements, design

A double-blind randomized controlled trial was carried out. Participants took 3 g fish oil or olive oil (placebo) per day, provided in three soft-gel capsules, for 4 weeks. The fish oil contained 2.3 g of n-3 PUFA (1.74 g EPA, 0.25 g DHA). The dose and duration were chosen on the basis of a consensus paper (Freeman, *et al.*, 2006), and was comparable with Fontani, *et al.* (2005). Both the fish-oil and placebo capsules were lemon-flavoured to maintain the blind. Randomization was carried out in blocks of six. The study was approved by the Medical Ethics Committee of Leiden University Medical Center.

Measures

Interview and self-report measures The expanded version of the *Mini International Neuropsychiatric Interview* (M.I.N.I. PLUS; Sheehan and Lecrubier, 2006) was used to assess current and lifetime DSM-IV diagnoses. The *Beck Depression Inventory – II* (BDI – II; Beck, *et al.*, 1988; Van der Does, 2002b) was used to measure symptoms of depression during the past 2 weeks. Mood states during the past week were measured with the shortened *Profile of Mood States* (POMS) (McNair, *et al.*, 1971; Wald and Mellenbergh, 1990). The subscales are sadness, anger, fatigue, tension and vigour. Personality traits were assessed with the *Behavioral Inhibition/Behavioral Activation Scales* (BIS/BAS) scales (Carver and White, 1994). The BIS is sensitive to signals of punishment, non-reward and novelty and the BAS has three subscales: Drive, Fun Seeking and Reward Responsiveness. Finally, cognitive reactivity was mea-

sured with the *Leiden Index of Depression Sensitivity – Revised* (LEIDS-R; Van der Does, 2002a; Van der Does and Williams, 2003). The subscales are Hopelessness/Suicidality (HOP); Acceptance/Coping (ACC); Aggression (AGG); Control/Perfectionism (CTR); Risk Aversion (RAV) and Rumination (RUM). The scale has been found to discriminate between never-depressed and recovered-depressed individuals (Van der Does, 2002a; Merens, *et al.*, 2005) and predicts response to serotonin challenge (Booij and Van der Does, 2007).

Neutral and emotional information processing tests An *Affective Go/No-Go task* was used, consisting of eight blocks of 16 stimuli. In each block, single words were presented with a stimulus interval of 1200 ms. Half of the stimuli were positive words (e.g., happy, clean) and half were negative (e.g., sad, guilt). In each block, either the positive (P) or negative (N) words were designated ‘targets’. The blocks were presented in a NNPPNNPP or PPNPPNN order. Because of this arrangement, three blocks were ‘shift’ blocks, where participants had to withhold responding to stimuli that were targets in the previous block, and four blocks were ‘non-shift’ blocks.

An *Attentional Go/No-Go task* was also used, with parameters identical to the Affective Go/No-Go task, except that letters and digits were used as targets.

The *Facial Expression Recognition Task* features five basic emotions (happiness, sadness, fear, anger and disgust) taken from the Pictures of Facial Affect Series (Ekman and Friesen, 1976). A male and female examples of these pictures were morphed between each prototype and neutral in 10% steps. Four trials of each emotion were presented at each intensity level. These stimuli were presented in randomized order for 500 ms and replaced by a blank screen. Participants were asked to respond as quickly and accurately as possible.

The *15 words test* was used to assess memory performance (Saan and Deelman, 1986). A list of 15 unrelated, neutral words was presented from tape. Immediate recall was tested after each of the two consecutive presentations. Participants continued with non-verbal tasks and after 20 min delayed recall was tested. Parallel versions were used at each session in a randomized sequence. Immediate recall was defined as the total of correct words at the second trial; consolidation as the number of correct words at delayed recall compared with the second trial.

The *Decision-making (gambling) task* measures decision-making behaviour over a variety of differentially weighted contingencies and also distinguishes risk-seeking from risk-averse behaviour. It has been previously described in detail by Rogers, *et al.* (2003). On each trial, participants were asked to choose between two simultaneously presented gambles. Each gamble was visually represented by a histogram, the height of which indicated the probability of gaining a given number of points (see Figure 1A). The possible gains were indicated in green ink above the histogram and the potential losses were indicated in red ink below the histogram. One gamble (coloured yellow) was always the control gamble, which had a 0.50 probability of winning 10 points and a 0.50 chance of losing 10 points

(with an expected value of 0). The alternative ‘experimental’ gamble (coloured blue) varied in the probability of winning, which was either high or low (0.60 vs 0.40), possible gains, which were either large or small (70 vs 30 points) and possible losses, which were either large or small (70 vs 30 points). These variables were crossed to produce eight trial types in which the expected value of the ‘experimental’ gamble varied between –20 and +20. The control and the ‘experimental’ gamble appeared randomly on the left or right. The participants indicated their choice by a key press. Dependent measures were the proportion of choices of the ‘experimental’ over the control gamble as a function of its probability of winning, size of possible gains and the size of possible losses and the mean deliberation time (ms) for these choices.

Two extra trial types were also included that represented choices between gambles known to be subject to the non-normative biases of risk-aversion and risk-seeking choices and that cannot be explained by decision-makers choosing actions that maximize expected value (the ‘reflection effect’; see Kahneman and Tversky, 1979). The first such trial type was a ‘gains-only’ trial in which the participants were presented with a choice between a guaranteed win of 30 points versus a 0.5 chance of winning 60 points and a 0.5 chance of losing 0 points (see Figure 1B). Neither option had any associated losses. On the contrary, the second trial type was a ‘losses-only’ trial in which the participants were presented with a choice between a guaranteed loss of 30 points versus a 0.5 chance of losing 60 points and a 0.5 chance of losing 0 points. Neither option had any potential gains. Within both the ‘gains-only’ and ‘losses-only’ trial types, the expected value of each gamble was equal; however, decision-makers usually exhibit risk-aversion in the former case but risk-seeking behaviour in the latter case (Schneider and Lopes, 1986).

The 10 trial types were presented pseudorandomly within four blocks of 20 trials. Across the four blocks, there were eight repetitions of each ‘experimental’ gamble and eight repetitions of each of the ‘gains-only’ and the ‘losses-only’ trial types. To maintain participants’ interest in the test, a monetary reward of 1 eurocent was placed on each point. Participants started off each block of trials with 100 cents and could keep the average gain of all the blocks (total gains were typically around 3 euros). Visual feedback was given after each choice and the revised points total was presented for 2 s before the next trial. At the end of each block, the participants were given a final score for that block. This task was administered only at post-treatment, as previous pharmacological studies of decision-making involving within-subject designs have found significant order effects (Wood, *et al.*, 2006).

Manipulation check

Immediately before and after treatment, fatty acid composition was determined in plasma to measure compliance and to detect possible increased fish or fish oil consumption in the placebo group. Venous blood was obtained (10 mL) in sodium heparin

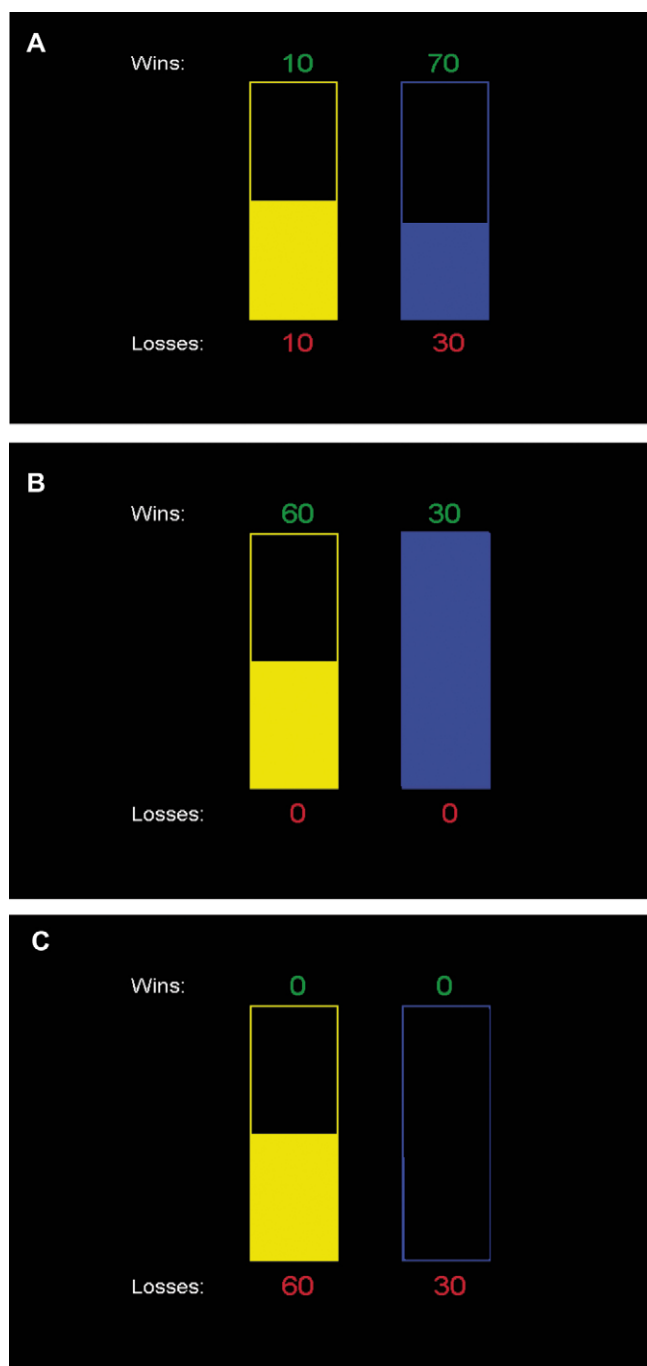


Figure 1 An example visual display from the decision-making task, consisting of an 'experimental' gamble with a 0.40 chance of winning 70 points and a 0.60 probability of losing 30 points versus the control gamble with a 0.50 chance of winning 10 points and a 0.50 of losing 10 points (Panel A). A 'Gains only' trial consisting of a certain win of 30 points and a gamble with a 0.50 probability of winning 60 points or 0 points (Panel B). A 'Losses only' trial consisting of a certain loss of 30 points and a gamble with a 0.50 probability of losing 60 points or 0 points (Panel C).

tubes. After sampling, it was immediately placed on ice and centrifuged for 20 min at $2650 \times g_{\max}$. The samples were stored at -80°C until analysis by gas chromatography. Self-reported compliance and the success of blinding were checked by a questionnaire after completion of the study.

Procedure

Participants were recruited through advertisements. The screening session started with the informed consent procedure, followed by the M.I.N.I.-PLUS and a check of the other inclusion and exclusion criteria. In a subsequent session a few days later, blood sample was taken, and the questionnaires and tests were administered (t_0). The intake of supplements started the same day. The cognitive tests and the POMS were re-administered 1 week later (t_1), and 4 weeks later the same procedure was repeated as at pre-treatment with the addition of the decision-making task (t_4). All measurements were taken at the same time of the day for each participant. Participants received 45 euros.

Statistical analysis

The questionnaires and the cognitive tasks were analysed using repeated measures analysis of variance (rmANOVA), with treatment group (n-3 PUFAs versus placebo) as between-subjects factor and time (t_0 , t_1 , t_4) as within-subjects factor. Data of the Affective Go/No-Go task were analysed with additional within-subject factors target valence and shift condition. Dependent measures for the Go/No-Go tasks were omissions (failures to respond to targets), false alarms and reaction times for hits. For the emotion recognition task, accuracy data were analysed with rmANOVA for each emotion separately (with time and intensity as within-subject factors). Reaction times were examined with emotion and time as within-subject factors. The 15-words list task had recall condition (immediate versus delayed) as additional within-subject factor. For the decision-making task, the proportionate choice data and mean deliberation times were analysed with rmANOVAs with between-subject factor of treatment, and within-subject factors of probability of winning, size of possible gains and size of possible losses. The 'gains-only' and 'losses-only' trials were analysed with rmANOVA with between-subject factor of treatment and within-subject factor of trial type.

Results

Data screening

Before analysis, all data were examined for accuracy of data-entry, missing values and normal distribution assumptions. Scores were normally distributed after square root transformations were performed on the POMS subscales, on the HOP

subscale of the LEIDS-R and on the misses and false alarms of the attentional and affective go/no-go tasks (tables show untransformed values). Extreme outlying data ($z > |3|$) were removed from the analysis if not corrected by transformations. For the decision-making task, proportionate choice data were arcsine-transformed.

Participant characteristics

Fifty-four of the 56 participants completed the study. The two dropouts stopped soon after the first measurement: one because of having difficulty swallowing the capsules and the second one because of personal problems unrelated to the experiment. These participants were not included in the analyses. The demographic characteristics of the participants are shown in Table 1. No group differences were found with respect to age, gender ($\chi^2(1) = 0.9$; $P > 0.05$), vitamin use ($\chi^2(2) = 2.9$; $P > 0.05$), BMI ($t(52) = 0.20$; $P > 0.05$) and previous psychiatric diagnoses ($\chi^2(5) = 4.0$; $P > 0.05$). There were also no baseline group differences on the BDI-II, the BIS-BAS, the LEIDS-R and POMS subscales (all $P > 0.05$).

Table 1 Baseline sample characteristics (means \pm SD)

	Treatment condition	
	Omega-3 <i>N</i> = 27	Placebo <i>N</i> = 27
Females	<i>n</i> = 21	<i>n</i> = 23
Age in years	22.2 \pm 3.6	22.6 \pm 4.1
BMI (kg/m ²)	21.9 \pm 2.3	21.8 \pm 2.2
Previous psychiatric diagnoses		
No diagnosis	<i>n</i> = 22	<i>n</i> = 21
Depression	<i>n</i> = 4	<i>n</i> = 4
Hypomania	<i>n</i> = 1	<i>n</i> = 0
Panic disorder	<i>n</i> = 0	<i>n</i> = 1
Agoraphobia	<i>n</i> = 0	<i>n</i> = 1
Depressive symptoms (BDI-II)	4.1 \pm 4.7	5.8 \pm 5.2
Behavioural activation		
Drive	11.5 \pm 2.0	11.6 \pm 1.9
Fun seeking	11.9 \pm 2.2	11.3 \pm 1.7
Reward responsiveness	17.2 \pm 1.6	16.4 \pm 2.3
Behavioural inhibition	18.6 \pm 3.5	19.7 \pm 2.9
LEIDS-R baseline	28.93 \pm 13.38	32.00 \pm 11.71
POMS baseline		
Fatigue	2.85 \pm 2.48	3.81 \pm 3.14
Anger	2.15 \pm 2.21	2.93 \pm 2.16
Tension	2.44 \pm 2.22	3.81 \pm 3.88
Sadness	2.07 \pm 2.97	2.26 \pm 2.85
Vigour	8.63 \pm 3.28	9.74 \pm 3.38

BMI, body mass index; BDI-II, Beck Depression Inventory, 2nd edition.

Compliance and manipulation check

Biochemical analyses of n-3 PUFAs concentrations in plasma level showed no differences at baseline between the two groups for EPA ($t(45) = 0.92$; $P > 0.05$), and for oleic acid ($t(45) = 1.38$; $P > 0.05$), but a small difference was found between the two groups in DHA concentrations ($t(45) = 2.08$; $P < 0.05$), with the placebo group having a lower mean concentration. There was a significant interaction effect of treatment \times time for EPA [$F(1,45) = 77.04$, $P < 0.001$] and DHA [$F(1,45) = 24.86$, $P < 0.001$]. The n-3 PUFA group showed a significant increase of EPA ($t(21) = 8.75$; $P < 0.001$) and of DHA ($t(21) = 7.55$; $P < 0.001$) ($\eta^2 = 1.5$). The placebo group had equal EPA concentrations before and after treatment ($t(24) = 1.53$; $P > 0.05$) but a small increase in DHA ($t(24) = 2.43$; $P < 0.05$) ($\eta^2 = 0.35$) (See Table 2). Self reported compliance was excellent and there was no difference between the two groups ($\chi^2(4) = 1.78$; $p > 0.05$). However, examination of individual changes of fatty acids concentrations revealed five outliers. Three participants in the n-3 PUFA group had a very low increase of EPA concentrations ($\Delta\text{EPA} < 0.6$, $z < -1.4$), whereas two participants in the placebo group showed a higher than expected increase in EPA ($\Delta\text{EPA} > 0.45$, $z > 1.8$). Like the two early drop-outs, we excluded these participants from analyses since the aim of this study was to investigate the effects of raised EPA concentrations on cognition in healthy volunteers. In other words, we did not do an intention-to-treat analysis since the study was not designed as a therapeutic trial but as a challenge study.

The blinding procedure was quite successful. Nine out of 27 participants who were randomized to the n-3 PUFA group and ten participants in the placebo group correctly guessed their group allocation. Nine participants in the n-3 PUFA group and four participants in the placebo group guessed wrongly, and 21 participants had no idea about treatment assignment. There were no differences between the groups in terms of expectancy ($\chi^2(2) = 3.15$; $p > 0.05$).

Mood states

Separate rmANOVAs on each subscale showed no significant time \times treatment interactions for all subscales, except for the fatigue subscale [$F(2,94) = 3.19$; $P = 0.046$]. Between-group comparisons at separate time points showed a trend at t_0 ($P = 0.07$), a non-significant difference at t_1 and a significant difference after 4 weeks of supplementation, ($P = 0.03$). Mean scores are presented in Table 3.

Cognition

Cognitive reactivity The time \times treatment interaction was significant for the Control/Perfectionism subscale of the LEIDS-R [$F(1,47) = 4.95$; $P = 0.03$]. The change over time was significant for the n-3 PUFA group ($t(23) = 2.66$;

Table 2 Plasma fatty acids concentrations as percentage of total fatty acids (mean \pm SD)

	Omega-3		Placebo		Interaction effect
	Pre	Post	Pre	Post	
EPA	0.51 \pm 0.21	2.84 \pm 1.26	0.45 \pm 0.21	0.52 \pm 0.24	$P < 0.001$
DHA	1.93 \pm 0.45	2.60 \pm 0.58	1.69 \pm 0.36	1.81 \pm 0.34	$P < 0.001$
Oleic Acid	17.85 \pm 1.93	16.79 \pm 1.62	18.76 \pm 2.50	20.04 \pm 2.36	$P = 0.001$

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 3 Mood states and cognitive reactivity before and after supplementation

	Omega-3 (<i>n</i> = 24)		Placebo (<i>n</i> = 25)		Interaction effect
	Pre	Post	Pre	Post	
POMS					
Fatigue	2.5 ± 2.3	2.0 ± 2.3	3.6 ± 2.8	3.6 ± 3.3	<i>F</i> (2,94) = 3.19; <i>P</i> = 0.046
Anger	1.8 ± 1.6	1.8 ± 2.1	2.8 ± 2.1	2.4 ± 2.5	<i>F</i> (2,94) = 0.24; <i>P</i> = 0.79
Tension	2.3 ± 2.1	2.0 ± 1.6	3.5 ± 3.7	2.8 ± 2.5	<i>F</i> (2,94) = 0.43; <i>P</i> = 0.65
Sadness	1.8 ± 2.7	1.6 ± 2.8	1.9 ± 2.3	1.8 ± 2.6	<i>F</i> (2,94) = 0.09; <i>P</i> = 0.91
Vigour	8.3 ± 3.1	9.7 ± 4.3	9.5 ± 3.3	10.0 ± 4.3	<i>F</i> (2,94) = 0.25; <i>P</i> = 0.78
LEIDS – R					
RAV	7.6 ± 3.9	6.5 ± 3.7	7.9 ± 3.4	9.0 ± 4.0	<i>F</i> (1,47) = 3.05; <i>P</i> = 0.09
HOP	2.5 ± 2.7	2.2 ± 1.4	2.5 ± 1.8	3.3 ± 2.4	<i>F</i> (1,47) = 2.22; <i>P</i> = 0.14
CTR	5.5 ± 3.5	4.5 ± 3.0	6.7 ± 3.3	7.2 ± 4.0	<i>F</i> (1,47) = 4.95; <i>P</i> = 0.03
ACC	1.0 ± 1.2	1.1 ± 1.4	1.5 ± 2.1	1.7 ± 2.1	<i>F</i> (1,47) = 0.03; <i>P</i> = 0.85
AGG	4.7 ± 4.1	4.2 ± 4.0	4.5 ± 3.6	4.4 ± 3.7	<i>F</i> (1,47) = 0.34; <i>P</i> = 0.56
RUM	7.0 ± 3.7	6.8 ± 3.7	9.3 ± 3.7	9.6 ± 4.0	<i>F</i> (1,47) = 0.27; <i>P</i> = 0.60
Total	28.3 ± 13.6	25.3 ± 12.3	32.4 ± 12.1	35.1 ± 14.1	<i>F</i> (1,47) = 3.68, <i>P</i> = 0.06

Means \pm standard deviations.

POMS, Profile of Mood States; LEIDS – R, Leiden Index of Depression Sensitivity – Revised; HOP, Hopelessness/Suicidality; ACC, Acceptance/Coping; AGG, Aggression; CTR, Control/Perfectionism; RAV, Risk Aversion; RUM, Rumination.

$P = 0.01$) but not for the placebo group ($P > 0.05$). Trends were found for the time \times treatment interaction for the Risk Aversion subscale [$F(1,47) = 3.05$; $P = 0.087$] and the total LEIDS-R score [$F(1,47) = 3.68$; $P = 0.06$] (See Table 3). The change of EPA concentrations over time (Δ EPA) correlated significantly with change of LEIDS-R-total ($r = 0.35$, $P = 0.02$) and with the change of Control/Perfectionism ($r = 0.30$, $P = 0.04$). No other significant correlations were found between Δ EPA and LEIDS-R subscale change scores.

Emotional and neutral information processing

For both the *Attentional* and *Affective Go/No-Go tests*, analyses showed no significant interaction effects of time \times treatment for the number of omissions, the number of false alarms and for reaction times. The data of both tests are summarized in Table 4. For *Facial Emotion Recognition* accuracy, all time \times intensity \times treatment interactions were non-significant and no significant effects on reaction times were found

($P > 0.05$). On the *15 word list (memory) test*, there were also no significant time \times treatment interactions for immediate recall, delayed recall and consolidation ($P > 0.05$).

Decision making

Proportionate choice and deliberation times Participants chose the experimental gamble significantly more often when the probability of winning was high compared with when it was low [$F(1,47) = 143.83$; $P < 0.001$]; when the expected gains were large compared with when they were small [$F(1,47) = 63.09$; $P < 0.001$] and less often when expected losses were large compared with when they were small [$F(1,47) = 99.69$; $P < 0.001$]. There were no significant two-way interactions between probability or size of gains or size of losses and treatment ($P > 0.05$). There were also no significant three- or four-way interactions between treatment group, probability and size of gains or losses ($F < 1$; $P > 0.05$). Deliberation times were not affected by the probability of winning,

Table 4 Means (\pm SD) of misses, false alarms and mean reaction time on the attentional and affective go/no-go tasks

	Omega-3		Placebo	
	Pre	Post	Pre	Post
<i>Attentional Go/no-go</i>				
Misses	1.4 \pm 3.3	0.7 \pm 1.3	2.9 \pm 4.6	1.4 \pm 2.5
False alarms	2.7 \pm 3.5	1.4 \pm 1.6	3.1 \pm 4.1	1.7 \pm 2.5
Reaction time	401.4 \pm 37.3	408.5 \pm 43.6	417.4 \pm 50.9	408.1 \pm 42.9
<i>Affective Go/no-go</i>				
Negative valence				
Misses	2.3 \pm 3.0	2.1 \pm 2.8	1.7 \pm 1.6	1.3 \pm 1.3
False alarms	1.9 \pm 2.5	1.4 \pm 2.0	1.2 \pm 1.3	1.3 \pm 1.7
RT's	580.1 \pm 63.6	581.9 \pm 54.3	609.1 \pm 84.2	582.1 \pm 70.4
Positive valence				
Misses	2.7 \pm 3.4	2.4 \pm 4.1	1.8 \pm 1.9	1.4 \pm 2.3
False alarms	1.2 \pm 1.1	1.2 \pm 1.1	1.6 \pm 1.4	1.5 \pm 1.8
RT's	590.3 \pm 57.3	590.5 \pm 64.3	602.9 \pm 81.1	586.2 \pm 58.3
Shift blocks				
Misses	1.8 \pm 2.9	1.3 \pm 2.1	1.0 \pm 1.0	1.3 \pm 2.1
False alarms	1.4 \pm 1.3	1.0 \pm 1.5	1.5 \pm 1.1	1.5 \pm 1.9
RT's	576.7 \pm 55.2	579.6 \pm 51.8	591.8 \pm 73.6	589.2 \pm 67.3
Non-shift blocks				
Misses	2.4 \pm 2.4	2.8 \pm 4.1	2.0 \pm 1.9	1.4 \pm 1.5
False alarms	1.4 \pm 1.6	1.4 \pm 1.7	1.2 \pm 1.4	1.2 \pm 1.5
RT's	586.8 \pm 65.2	592.3 \pm 64.7	608.7 \pm 88.6	585.9 \pm 64.4

Table 5 Proportionate choices and mean deliberation times (\pm SEM) as a function of probability of winning, levels of expected gains and expected losses and treatment group, in the decision-making task

	Probability of winning		Expected gains		Expected losses	
	High	Low	Large	Small	Large	Small
Proportionate choice						
Omega 3	0.72 \pm 0.04	0.24 \pm 0.05	0.57 \pm 0.04	0.39 \pm 0.03	0.35 \pm 0.04	0.60 \pm 0.04
Placebo	0.74 \pm 0.04	0.21 \pm 0.05	0.56 \pm 0.04	0.39 \pm 0.03	0.36 \pm 0.04	0.59 \pm 0.03
Reaction times						
Omega 3	2461 \pm 259	2469 \pm 214	2393 \pm 232	2538 \pm 223	2474 \pm 216	2457 \pm 240
Placebo	2665 \pm 254	2546 \pm 209	2582 \pm 227	2628 \pm 219	2555 \pm 212	2655 \pm 235

size of expected gains or size of expected losses ($P > 0.05$). There were no significant interactions involving treatment group and any of these conditions [$F < 1$; $P > 0.05$]. In each condition, the n-3 PUFA group was faster than the placebo group in placing their gambles; however, this difference was not statistically significant [$F(1,47) = 0.19$; $P > 0.05$]. Data are presented in Table 5.

"Gains only" and "Losses only" trials: risk-seeking behaviour and deliberation times As expected, participants took significantly fewer risks in gains-only trials than in losses-only trials [$F(1,47) = 128.92$; $P < 0.001$]. On approximately 80% of trials, participants preferred the certain gain of 30 cents over playing a gamble with a 0.5 probability of winning 60 cents or nothing. This effect was significantly modulated by treatment with n-3

PUFAs [$F(1,47) = 6.51$; $P = 0.01$]. In the 'gains only' trials, the n-3 group showed more risk-seeking decision-making behaviour than the placebo group, $t(47) = 2.32$, $P = 0.03$ (see Figure 2). In losses-only trials, participants almost always rejected the guaranteed loss of 30 cents and preferred to play a gamble with a 0.5 chance of losing nothing or losing 60 cents; there were no differences between treatment groups ($P > 0.05$). Participants made significantly faster choices on the 'gains-only' trials in comparison with the 'losses-only' trials [$F(1,47) = 46.35$; $P < 0.001$]. The n-3 PUFA group was approximately 500 ms slower than the placebo group in the gains-only trials and about 200 ms slower in the losses-only trials; however, this difference between the two groups was non-significant [$F(1,47) = 0.51$; $P > 0.05$], and neither was the treatment \times trial type interaction [$F(1,47) = 0.19$; $P > 0.05$].

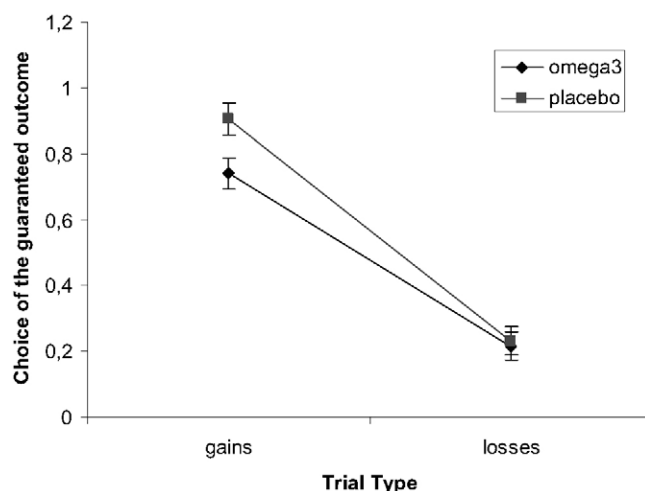


Figure 2 Proportion of choices of the guaranteed outcome in the 'gains-only' and 'losses-only' trials by the omega-3 group and placebo, in the decision-making task.

Discussion

This study showed no effects of n-3 PUFA supplementation in healthy volunteers on attention, memory, response inhibition and emotion recognition. However, selective effects were observed on cognitive reactivity and risky decision-making. As expected, there were no effects on self-reported mood, except for a small effect on fatigue that should be interpreted with caution because at baseline the groups differed at the trend level of significance. The results on the POMS are in contrast with Fontani, *et al.* (2005), who found an increase in vigour and a decrease in all other mood states (anger, anxiety, depression, fatigue). The difference between studies might be due to the fact that supplements in Fontani, *et al.* contained more DHA (0.8 g/day versus 0.25 g/day in the present study). However, EPA seems to have stronger effects on mood than DHA (Lin and Su, 2007), and these dosages were comparable. As the participants in the Fontani, *et al.* study were regular exercisers, an interaction between n-3 PUFA supplementation and physical exercise may also have occurred. As noted in the introduction, we cannot be sure whether the effects in Fontani, *et al.* (2005) were actually larger than placebo.

No significant differences were found between the two groups in the number of omissions, false alarms and in reaction times in the attentional go/no-go task involving neutral material. This is also in contrast to Fontani, *et al.* (2005) who found an effect on reaction times. A major difference between the two studies is that, on closer look, the tasks presented as Go/No-Go tasks by Fontani, *et al.* involved significant elements of vigilance and short-term memory, but do not seem to measure response inhibition. No effect of n-3 PUFA supplementation was found on any of the outcome measures in the Affective

Go/No-Go task. In healthy samples, tryptophan depletion, which reduces serotonergic function, resulted in slower responses to happy but not to sad words (Murphy, *et al.*, 2002). Tyrosine depletion, which reduces dopaminergic function, was also associated with a sad latency bias (McLean, *et al.*, 2004). Apparently, any effects of n-3 PUFA supplementation on serotonergic or dopaminergic neurotransmission are too small to affect attention and response inhibition (in relation to both neutral or valenced stimuli) in healthy individuals. Of course, there may also have been too little room for improvement (ceiling effect). The same applies to the measures of facial emotion recognition and memory, which were also not affected by n-3 PUFA supplementation. These measures were included because they had been shown to be sensitive to various short-term manipulations of neurotransmitters (Harmer, *et al.*, 2003; Attenburrow, *et al.*, 2003; Riedel, *et al.*, 1999; Schmitt, *et al.*, 2000).

An effect of n-3 PUFAs on cognitive reactivity was observed only on the Control/Perfectionism subscale of the LEIDS-R, where the n-3 PUFA group showed decreased reactivity. This scale measures the extent to which a mild dysphoric mood triggers perfectionistic thoughts. This effect, along with a trend-level effect on the total score, correlated with the change of EPA and seems worthy of follow-up in future research.

The two groups showed similar patterns of decision-making in their choices of the experimental gamble over the control gamble across almost all conditions. Deliberation times were also similar. Previously, Rogers, *et al.* (2003) found that participants who underwent tryptophan depletion – which reduces central serotonin activity – showed reduced discrimination between the magnitudes of expected gains compared with non-depleted participants. It does not necessarily follow that opposite interventions would improve the discrimination between the magnitude of expected gains. However, it is also possible that the effects of n-3 PUFAs on serotonin function, if any, are too small to alter such aspects of decision-making.

However, n-3 PUFA-treated participants showed less risk-aversion in the face of certain gains. Previous studies with the risky decision-making task have not found effects on such non-normative aspects of decision-making. Thus, the reflection effect was consistently found, with no differences between treatment groups, following tryptophan depletion (Rogers, *et al.*, 2003), the β -blocker propranolol (Rogers, *et al.*, 2004), tyrosine depletion (Scarna, *et al.*, 2005) and tetrahydrocannabinol (Rogers, *et al.*, 2007). It should be noted that each of these studies used a version of the task in which the differences between the largest and the smallest gains, losses and probabilities were all greater than those of the version used in the present study. The present version required participants to make finer discriminations between different levels of reinforcement when making choices, possibly improving the task's sensitivity. Consistent with this possibility, we have found similar changes in the reflection effect following 2 weeks of treatment with tryptophan as a dietary supplement, and following single doses of duloxetine (Rogers, *et al.*, unpublished article).

In the present experiment, the effects of n-3 PUFAs were expressed as increased risk-seeking behaviour compared with treatment with placebo. Because this finding was accompanied by longer deliberation times, we suggest that this difference does not reflect impulsiveness but rather a willingness to take calculated risks. The lack of effects on the two Go/No-Go tasks also argues against an explanation in terms of elevated impulsivity. Previous research on risk-taking tendencies and mood suggests that under a low-risk situation a positive mood may induce more risky decision-making in a gambling task, as the likely gain may enhance or at least maintain the positive mood (Isen and Geva, 1987). In the gains-only trials of the gambling task, choosing the guarantee-gain gamble presumably requires less mental effort expenditure than when choosing the risky gamble. Meijman, *et al.* (1992; as cited in Hockey, *et al.*, 2000) found that people under fatigue put less mental effort on cognitive probe tasks and may prefer activities requiring less effort and avoid engaging in high-level control actions. This may be related to lower reported fatigue of the n-3 PUFA group on the POMS. These results seem to indicate that participants were more willing to put more mental effort in their decision-making choices and eventually were more willing to engage in risky behaviour.

To our knowledge this was the first study to examine the effects of n-3 PUFA supplements on depression-related cognitive functioning in healthy individuals. By investigating a healthy sample, we could circumvent some potential confounders such as illness chronicity and concomitant medications. Although we found few effects of n-3 PUFAs on cognition, the relatively short duration of the intervention, the initial high levels of DHA in the n-3 PUFA group and the change in DHA levels in the placebo group may all have masked other effects of the manipulation. The sample size was large enough to detect moderately large effects with the conventional level of statistical power (0.8); hence, subtler effects may have gone undetected. However, the positive findings should also be considered with caution because we made a large number of comparisons between groups. Finally, it should be noted that the decision-making task was only administered at post-test, which means that the difference in the gains-only trials cannot be confidently attributed to treatment. A logical next step in research would be to examine cognitive effects of n-3 PUFAs in depression-vulnerable samples, characterized by history of depression, family history of depression or genotype.

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References

Appleton, KM, Hayward, RC, Gunnell, D, Peters, TJ, Rogers, PJ, Kessler, D, *et al.* (2007) Effects of n-3 long-chain polyunsaturated

fatty acids on depressed mood: systematic review of published trials. *Am J Clin Nutr* 84: 1308–1316.

- Attenburrow, MJ, Williams, C, Odontiadis, J, Reed, A, Powell, J, Cowen, PJ, *et al.* (2003) Acute administration of nutritionally sourced tryptophan increases fear recognition. *Psychopharmacology* 169: 104–107.
- Austin, MP, Mitchell, P, Goodwin, GM (2001) Cognitive deficits in depression: possible implications for functional neuropathology. *Br J Psychiatry* 178: 200–206.
- Beck, AT, Steer, RA, Garbin, MG (1988) Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin Psychol Rev* 8: 77–100.
- Booij, L, Van der Does, AJW (2007) Cognitive and serotonergic vulnerability to depression: convergent findings. *J Abnorm Psychol* 116: 86–94.
- Browning, M, Reid, C, Cowen, PJ, Goodwin, GM, Harmer, CJ (2007) A single dose of citalopram increases fear recognition in healthy subjects. *J Psychopharmacol* 21: 684–690.
- Carver, CS, White, TL (1994) Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS scales. *J Pers Soc Psychol* 67: 319–333.
- Chalon, S (2006) Omega-3 fatty acids and monoamine neurotransmission. *Prostaglandins Leukot Essent Fatty Acids* 75: 259–269.
- De la Presa, OS, Innis, SM (1999) Docosahexaenoic acid and arachidonic acid prevent a decrease in dopaminergic and serotonergic neurotransmitters in frontal cortex caused by α -linoleic and α -linolenic acid deficient diet in formula-fed piglets. *J Nutr* 129: 2088–2093.
- Ekman, P, Friesen, WV (1976) Measuring facial movement. *Environ Psychol Nonverbal Behav* 1: 56–75.
- Fontani, G, Corradeschi, F, Felici, A, Alfatti, F, Migliorini, S, Lodi, L (2005) Cognitive and physiological effects of omega-3 polyunsaturated fatty acid supplementation in healthy subjects. *Eur J Clin Invest* 35: 691–699.
- Freeman, MP (2000) Omega-3 fatty acids in psychiatry: a review. *Ann Clin Psychiatry* 12: 159–165.
- Freeman, PM, Hibbeln, JR, Wisner, KL, Davis, JM, Mischoulon, D, Peet, M, Keck, PE, Marangell, LB, Richardson, AJ, Lake, J, Stoll, AL (2006) Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. *J Clin Psychiatry* 67: 1954–1967.
- Hallahan, B, Garland, MR (2004) Essential fatty acids and their role in the treatment of impulsivity. *Prostaglandins Leukot Essent Fatty Acids* 71: 211–216.
- Hamazaki, T, Sawazaki, S, Kobayashi, M (1996) The effect of docosahexaenoic acid on aggression in young adults. A double-blind study. *J Clin Invest* 97: 1129–1134.
- Harmer, CJ, Bhagwagar, Z, Perrett, DI, Volm, BA, Cowen, PJ, Goodwin, GM (2003) Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology* 28: 148–152.
- Hibbeln, JR, Linnoila, M, Umhau, JC, Rawlings, R, George, DT, Salem, N (1998) Essential fatty acids predict metabolites of serotonin and dopamine in cerebrospinal fluid among healthy control subjects and early- and late-onset alcoholics. *Biol Psychiatry* 44: 235–242.
- Hockey, RGJ, Maule, JA, Clough, PJ, Bdzola, L (2000) Effects of negative mood states on risk in everyday decision making. *Cogn Emot* 14: 823–855.
- Isen, AM, Geva, N (1987) The influence of positive affect on acceptable level of risk and thoughts about losing: The person with a

- large canoe has a large worry. *Organ Behav Hum Decis Process* 39: 145–154.
- Kahneman, D, Tversky, A (1979) Prospect theory: an analysis of decision under risk. *Econometrica* 47: 263–292.
- Kodas, E, Galineau, L, Bodard, S, Vancassel, S, Guilloteau, D, Besnard, J, *et al.* (2004) Serotonergic neurotransmission is affected by n-3 polyunsaturated fatty acids in the rat. *J Neurochem* 89: 695–702.
- Lin, P-Y, Su, K-P (2007) A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. *J Clin Psychiatry* 68: 1056–1061.
- McLean, A, Rubinsztein, JS, Robbins, TW, Sahakian, BJ (2004) The effects of tyrosine depletion in normal healthy volunteers: implications for unipolar depression. *Psychopharmacology* 171: 286–297.
- McNair, DM, Lorr, M, Droppelamn, LF (1971) *Manual for the Profile of Mood States*. San Diego, CA: Educational and Industrial Testing Service.
- Merens, W, Booij, L, Markus, RC, Zitman, FG, Onkenhout, W, Van der Does, AJW (2005) The effects of a tryptophan-enriched diet on mood and cortisol response in unmedicated recovered depressed patients and controls. *Br J Nutr* 94: 415–422.
- Merens, W, Van der Does, AJW, Spinhoven, P (2007) The effects of serotonin manipulations on emotional information processing and mood. *J Affect Disord* 103: 43–62.
- Murphy, FC, Smith, KA, Cowen, PJ, Robbins, TW, Sahakian, BJ (2002) The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology* 163: 42–53.
- Parker, G, Gibson, NA, Brotchie, H, Heruc, G, Rees, A, Hadzi-Pavlovic, D (2006) Omega-3 fatty acids and mood disorders. *Am J Psychiatry* 163: 969–978.
- Riedel, WJ, Klaasen, T, Deutz, EPN, van Someren, A, van Praag, HM (1999) Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacology* 141: 362–369.
- Rogers, RD, Lancaster, M, Wakeley, J, Bhagwagar, Z (2004) Effects of beta-adrenoceptor blockade on components of human-decision making. *Psychopharmacology* 172: 157–164.
- Rogers, RD, Tunbridge, EM, Bhagwagar, Z, Drevets, WC, Sahakian, BJ, Carter, CS (2003) Tryptophan depletion alters the decision-making of healthy volunteers through altered processing of reward cues. *Neuropsychopharmacology* 28: 153–162.
- Rogers, RD, Wakeley, J, Robson, PJ, Bhagwagar, Z, Makela, P (2007) The effects of low doses of delta-9 tetrahydrocannabinol on reinforcement processing in the risky decision-making of young healthy adults. *Neuropsychopharmacology* 32: 417–428.
- Saan, RJ, Deelman, BG (1986) Nieuwe 15-woorden test A en B (15WTA en 15WTB) [New version of 15 Words Test (15WTA and 15WTB)]. In: Bauma, A, Mulder, J, Lindeboom, J, (eds), *Neuro-Psychologische Diagnostiek: Handboek* [Neuropsychological Diagnostics : Handbook]. Lisse: Swets & Zeitlinger Publishers. pp. 13–28.
- Scarna, A, McTavish, SFB, Cowen, PJ, Goodwin, GM, Rogers, RD (2005) The effects of a branched chain amino acid mixture supplemented with tryptophan on biochemical indices of neurotransmitter function and decision making. *Psychopharmacology* 179: 761–768.
- Schmitt, J, Jorissen, B, Sobczak, S, van Boxtel, MPJ, Hogervorst, E, Deutz, NEP, *et al.* (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. *Psychopharmacology* 14: 21–29.
- Schneider, SL, Lopes, LL (1986) Reflection in preference under risk: who and when may suggest why. *J Exp Psychol Hum Percept Perform* 12: 535–548.
- Sheehan, DV, Lecrubier, Y (2006) M.I.N.I. PLUS. Mini International Neuropsychiatric Interview. Version 5.0.0. <https://medical-outcomes.com>. Accessed June 10th, 2006.
- Sheehan, DV, Lecrubier, Y, Harnett-Sheehan, K, Amorim, P, Janavs, J, Weiller, E, *et al.* (1998) The Mini International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview. *J Clin Psychiatry* 59: 22–33.
- Van der Does, AJW (2002a) Cognitive reactivity to sad mood: structure and validity of a new measure. *Behav Res Ther* 40: 105–120.
- Van der Does, AJW (2002b) Handleiding bij de Nederlandse bewerking van de BDI-II. San Antonio, Texas/Amsterdam, The Netherlands: The Psychological Corporation/Harcourt Publishers.
- Van der Does, AJW, Williams, JMG (2003) The Leiden Index of Depression Sensitivity, Revised version (LEIDS-R). <http://www.dousa.nl/publications>. Accessed June 20th, 2006.
- Wald, FDM, Mellenbergh, GJ (1990) De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). *Ned Tijdschr Psychol* 45: 86–90.
- Wood, RM, Rilling, JK, Sanfey, AG, Bhagwagar, Z, Rogers, RD (2006) Effects of tryptophan depletion on the performance of an iterated Prisoner's Dilemma game in healthy adults. *Neuropsychopharmacology* 31: 1075–1084.
- Yehuda, S, Rabinovitz, S, Mostofsky, DI (1999) Essential fatty acids are mediators of brain biochemistry and cognitive functions. *J Neurosci Res* 56: 565–570.
- Young, G, Conquer, J (2005) Omega-3 fatty acids and neuropsychiatric disorders. *Reprod Nutr Dev* 45: 1–28.